Abstract
The isolated frog brainstem is an established preparation to quantify respiratory drive and the role of central CO2 sensitivity in control of breathing. Here, we establish a technique to quantify neuronal response to high CO2 (hypercapnia) and compare it to the concomitant response by the intact respiratory network. Recorded neurons were subsequently juxtacellularly labeled, facilitating identification of location and phenotype of respiratory CO2-sensitive neurons. Neurograms representing buccal and lung breathing were obtained with suction electrodes recording wholenerve activity in cranial nerves 5 and 7. Simultaneously, extracellular electrodes recorded single neurons in established CO2-sensitive sites of the frog brainstem. Neural activity was recorded while brainstems were exposed to normocapnia and hypercapnia (1.5 and 5.0% CO2, balance O2). Neurons were then entrained to an injected current and juxtacellularly labeled with biotinamide. After electrophysiology experiments, brainstem slices were stained with streptavidin-AlexaFluor and the recorded cells were imaged. Our results validate this technique, which is the first reported capacity to simultaneously assess CO2 sensitivity of the central network that controls breathing and a single neuron embedded in the network. We expect to use this technique to elucidate neural mechanisms responsible for respiratory drive.
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Simultaneous assessment of CO_2 sensitivity in the respiratory network and its neurons

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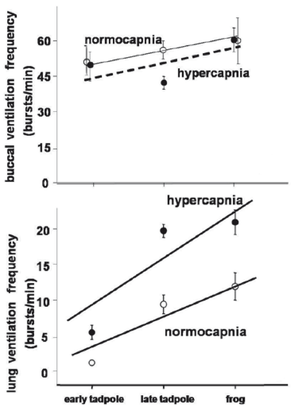
Introduction

Tadpoles exhibit bimodal breathing; a combination of buccal and lung breaths. Breathing is controled by a central neural network that is sensitive to changes in central CO_2. The respiratory neural network can be isolated as the en bloc brainstem preparation and remains active under physiologically relevant conditions for over 48h. Thus, the isolated tadpole brainstem preparation is a powerful tool for investigating neural control of breathing in vertebrates.

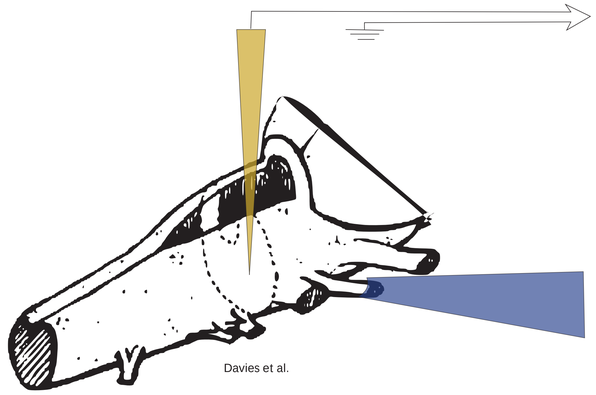


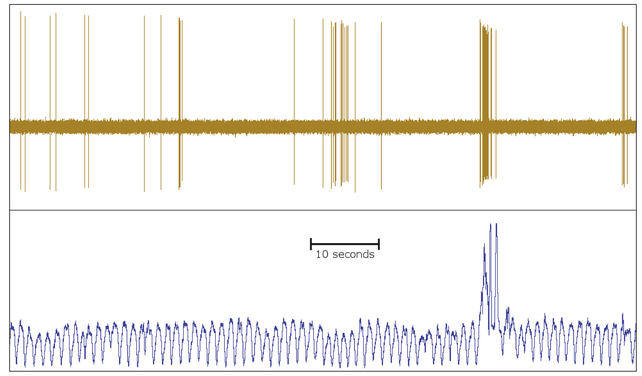
Aims

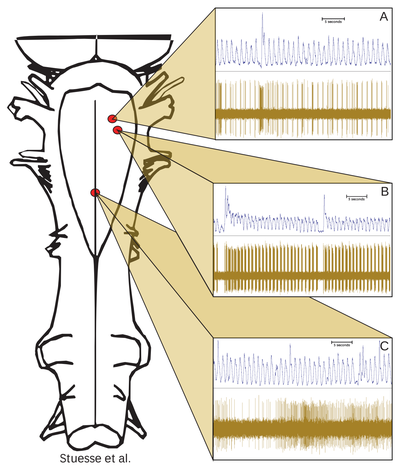
To simultaneously record respiratory-related activity from cranial nerves and single neurons
To label the recorded neuron
To characterize and quantify central chemosensitivity in the brainstem

Simultaneous Recordings

- Single-neuron and whole-nerve recordings are made simultaneously in vitro
- Neuron firing that is coincident with whole-nerve activity indicates respriratory-relatedness of the cell





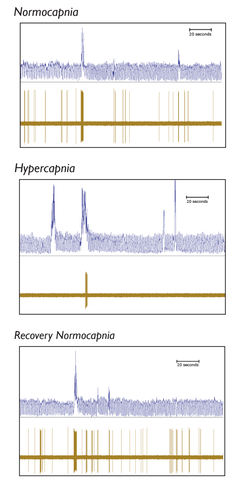


Chemosensitive Sites

- Single-neuon recordings were made in rostral (A & B) and caudal chemosensitive sites and the raphe (C)
- Firing of respiratory-related neurons (A & B) exhibits synchronicity to lung and/or buccal bursts
- CO_2-sensitive neurons, respiratory-related and unrelated, were found in all three chemosensitive sites

CO_2 Sensitivity

- CO_2-sensitive neurons changed firing frequency and pattern in response to hypercapnia
CO_2-induced firing changes included increases and decreases (see right) in frequency
CO_2-induced changes in pattern included increases (see right) and decreases in synchronicity



The novel technique of simultaneously recording cranial nerves and single neurons provides new insight into central CO_2 sensitivity.

Conclusions

Simultaneous recordings of single neurons and whole nerves, with subsequent labeling of the recorded cell provides a platform to ask questions about the functional relationships of respiratory-related neurons in central chemosensitivity.

Future Work

- Immunohistochemistry will be conducted to identify the neurotransmitter phenotype of respiratory-related, CO_2-sensitive neurons.
- Blockade of fast-synaptic transmission to identify neurons with intrinsic CO_2 chemosensitivity.
- Resolve temporal versus spatial recruitment of neurons contributing to chemoreceptor, motoneuron, and oscilattor neuron pools.

Methods

Whole-Nerve Recording

- The tadpole brainstem was isolated in a recording chamber and bathed in artificial cerebrospinal fluid (aCSF). Cranial nerve 5 or 7 was drawn into a suction electrode, which recorded whole-nerve respiratory activity.

Extracellular Recording

- A micro-electrode filled with 5% biotinamide was vertically inserted into the brainstem proximal to CN 5 or 10 or in the medullary raphe. Single-neuron activity was recorded and correlated with whole-nerve activity to determine its respiratory relatedness.

Treatments

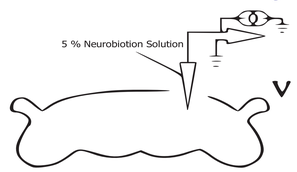
- The brainstem is bathed in normocapnia (1.5% CO_2) or hypercapnia (5% CO_2) aCSF.

Juxtacellular Labeling

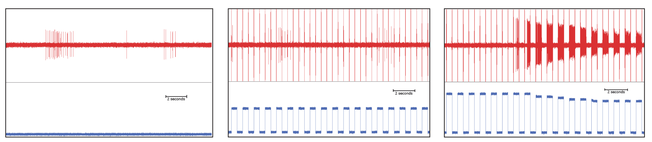
Neurons are entrained with juxtacellularn application of current pulses that induces electroporation of the cell membrane, and facilitates uptake of neurobiotin ejected from the microelectrode during the current pulse.

Tissue is fixed in 4% paraformaldehyde and then sliced into 60µm sections. Sections are stained with a streptavidin dye which binds to biotin, and imaged to visualize the recorded neuron.

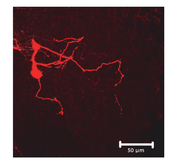
Juxtacellular Recording



Juxtacellular Entrainment



Juxtacellular Labeling



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